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A systematic review of East African-Indian family of *Mycobacterium tuberculosis* in Brazil

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ABSTRACT

Introduction: The *Mycobacterium tuberculosis* East African-Indian (EAI) spoligotyping family (belonging to lineage 1, Indo-Oceanic, defined by the region of deletion RD239) is distributed worldwide, but is more prevalent in Southeast Asia, India, and East Africa. Studies in Latin America have rarely identified EAI. In this study, we describe the occurrence of the EAI family in Brazil.

Methods: EAI was identified in a systematic literature review of genetic diversity studies pertaining to *M. tuberculosis* in Brazil, as well as in a survey conducted in Salvador, Bahia, located in the northeastern region of this country.

Results: The EAI6-BGD1 spoligotyping family and the EAI5 Spoligotype International Type (SIT) 1983 clade were the most frequently reported, with wide distribution of this particular clade described in Brazil. The distribution of other EAI spoligotyping patterns with broader worldwide distribution was restricted to the southeastern region of the country.

Conclusions: EAI may be endemic at a low frequency in Brazil, with some clades indicating increased fitness with respect to this population.

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Introduction

Tuberculosis (TB) remains one of the world deadliest communicable diseases.[1] *Mycobacterium tuberculosis* (Mtb) is broadly distributed across all five continents, although relatively few countries account for as much as 80% of all TB cases.[1] To date, seven lineages of Mtb complex have been recognized and associated with particular geographical regions.[2] There is evidence suggesting that these phylogeographic groups differ in their biological fitness and are best adapted to the sympatric human hosts.[3,4]

Three major spoligotyping families are most frequently found in Africa, Central America, Europe, and South America: Haarlem, Latin American-Mediterranean (LAM) and T from lineage 4 (which is defined by two deletions in the genome, one comprising the TbD1 region and another at the *pks15/1* locus).[2,5–7] The East African-Indian (EAI) spoligotyping family from lineage 1 (defined by the region of deletion RD239)[2,5–7] is prevalent in Southeast Asia, particularly in the Philippines, Myanmar, Malaysia, Vietnam, Thailand, India, and East Africa, yet is relatively rare in the Americas.[7]

EAI, a mildly virulent group, possesses a reduced potential for transmissibility.[8] Studies performed to characterize Mtb strains in circulation in Latin America have rarely identified EAI, and this family is considered to have limited distribution in comparison with other families in this region.[7] Here we report the detection of one case of the EAI family in Salvador, Bahia-Brazil, an area endemic for TB, and we review previous reports of the occurrence of this family in Brazil.

Methods

Recruitment and study design

A population-based study conducted in Salvador, the capital of the state of Bahia, from August 2008 to August 2010 involved sputum smear-positive patients in the context of an epidemiological survey that identified patients with pulmonary tuberculosis at local health clinics, with enrollment limited to one person per household (data not published). This is an endemic area where disease incidence was estimated at 62.7/100,000 in 2014, while the overall TB incidence in Brazil was reported to be 33.5/100,000.[9] A total of 362 mycobacterial isolates were obtained from the collected positive sputum smears, among which 351 isolates were successfully genotyped and one isolate was identified as belonging to the EAI family. We describe this finding herein and review the previous occurrence of EAI in the country. The present study (CAAE: 0016.0.069.000-07) was approved by the Ethics Committee of the Gonçalo Moniz Research Center (Fiocruz). It adhered to Resolution 196/96 established by the Brazilian National Health Council and complied with the Helsinki declaration guidelines.

Species identification and genotyping

Identification at the species level was performed by phenotypic and biochemical methods after culturing in Lowenstein-Jensen medium (Becton-Dickinson, Palo Alto, CA). RFLP was performed according with the method described

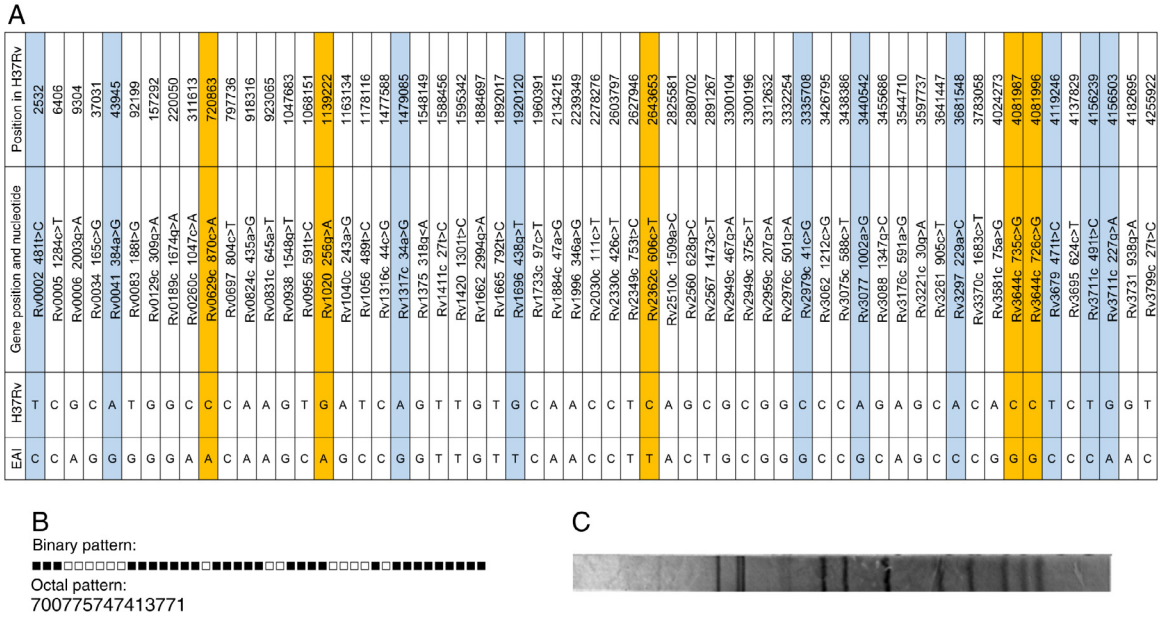

Fig. 1 – Genotypic profile of the isolate from Salvador, Bahia, Brazil assigned to the EAI6-BDG1 spoligotyping subfamily (SIT 702). (A) Single Nucleotide Polymorphisms (SNPs). Loci highlighted in blue show polymorphisms uniquely identified in the East African-Indian (EAI) strain as compared to the other 351 genotyped isolates in this series. Loci highlighted in orange show the SNPs that were also distinctive of EAI, according to Lopes et al.[12] (B) Spoligotyping pattern. (C) Restriction-fragment length polymorphism (RFLP).

Table 1 – Panel of 59 single nucleotide polymorphisms (SNPs) used for genotyping.

Genome location	Gene	Gene position and nucleotide	S or NS	Reference
2532	Rv0002	Rv0002_481t>C	S	13
6406	Rv0005	Rv0005_1284c>T	S	32
9304	Rv0006	Rv0006_2003g>A	NS	33
37031	Rv0034	Rv0034_165c>G	S	34
43945	Rv0041	Rv0041_384a>G	S	34
92199	Rv0083	Rv0083_188t>G	S	34
157292	Rv0129c	Rv0129c_309g>A	S	33
220050	Rv0189c	Rv0189c_1674g>A	S	34
311613	Rv0260c	Rv0260c_1047c>A	S	34
720863	Rv0629c	Rv0629c_870c>A	S	13
797736	Rv0697	Rv0697_804c>T	S	34
918316	Rv0824c	Rv0824c_435a>G	S	34
923065	Rv0831c	Rv0831c_645a>T	S	34
1047683	Rv0938	Rv0938_1548g>T	NS	35
1068151	Rv0956	Rv0956_591t>C	S	34
1139222	Rv1020	Rv1020_256g>A	NS	13
1163134	Rv1040c	Rv1040c_243a>G	S	34
1178116	Rv1056	Rv1056_489t>C	S	34
1477588	Rv1316c	Rv1316c_44c>G	NS	13
1479085	Rv1317c	Rv1317c_34a>G	NS	13
1548149	Rv1375	Rv1375_318G<A	S	34
1588456	Rv1411c	Rv1411c_27t>C	S	33
1595342	Rv1420	Rv1420_1301t>C	NS	13
1884697	Rv1662	Rv1662_2994G>a	S	34
1892017	Rv1665	Rv1665_792t>C	S	34
1920120	Rv1696	Rv1696_438g>T	NS	13
1960391	Rv1733c	Rv1733c_97c>T	NS	33
2134215	Rv1884c	Rv1884c_47a>G	S	33
2239349	Rv1996	Rv1996_346a>G	NS	33
2278276	Rv2030c	Rv2030c_111c>T	S	33
2603797	Rv2330c	Rv2330c_426c>T	S	33
2627946	Rv2349c	Rv2349c_753T>c	S	34
2643653	Rv2362c	Rv2362c_606c>T	S	13
2825581	Rv2510c	Rv2510c_1509a>C	S	36
2880702	Rv2560	Rv2560_628g>C	NS	34
2891267	Rv2567	Rv2567_1473c>T	S	34
3300104	Rv2949c	Rv2949c_467g>A	NS	33
3300196	Rv2949c	Rv2949c_375c>T	S	33
3312632	Rv2959c	Rv2959c_207g>A	NS	33
3332254	Rv2976c	Rv2976c_501g>A	S	13
3335708	Rv2979c	Rv2979c_41c>G	NS	13
3426795	Rv3062	Rv3062_1212c>G	S	13
3438386	Rv3075c	Rv3075c_588c>T	S	34
3440542	Rv3077	Rv3077_1002a>G	S	34
3455686	Rv3088	Rv3088_1347g>C	S	34
3544710	Rv3176c	Rv3176c_591a>G	S	34
3597737	Rv3221c	Rv3221c_30g>A	S	33
3641447	Rv3261	Rv3261_905c>T	NS	33
3681548	Rv3297	Rv3297_229a>C	S	13
3783058	Rv3370c	Rv3370c_1683c>T	S	34
4024273	Rv3581c	Rv3581c_75a>G	S	34
4081987	Rv3644c	Rv3644c_735c>G	S	13
4081996	Rv3644c	Rv3644c_726c>G	S	13
4119246	Rv3679	Rv3679_471T>c	S	34
4137829	Rv3695	Rv3695_624c>T	S	34
4156239	Rv3711c	Rv3711c_491t>C	NS	13
4156503	Rv3711c	Rv3711c_227g>A	NS	13
4182695	Rv3731	Rv3731_938g>A	NS	13
4255922	Rv3799c	Rv3799c_27t>C	S	34

S, synonymous; NS, non-synonymous.

by van Embden et al.[10] Spoligotyping profiles were obtained using the method established by Cowan et al.[11] and then submitted to the SITVIT WEB database for family and subfamily designation.[7] Single Nucleotide Polymorphisms (SNPs) were genotyped according to the method by Lopes et al.[12] using 59 SNPs located outside the genome regions known to be related to antibiotic resistance. The EAI lineage has been previously defined by the SNPs in Rv1020_256 and Rv2362c_606.[13]

Systematic review of the literature

We reviewed the studies of Mtb population genetics performed in Brazil to identify previous findings of EAI family tuberculosis isolates in the country. Published studies were located using the PubMed platform or the electronic libraries Scielo (Scientific Electronic Library Online Brazil) or BIREME (Virtual Health Library) through searches using the following terms: "*M. tuberculosis*" and (genotyping or spoligotyping) and Brazil, imposing no language restrictions. Studies were included in the analysis if fulfilling the following criteria: (i) Study reports more than 10 tuberculosis cases from Brazil; (ii) Study discriminates lineages of Mtb and identifies EAI family by spoligotyping or SNP; (iii) Population-based study published up to June 2016.

Designation of phylogenetic groups and genetic similarity analyses

The octal or binary spoligotyping patterns reported were retrieved from the articles obtained with this search and submitted (1) to the SITVIT WEB database for family and subfamily designation; and (2) to the MIRU-VNTR*plus* database to generate neighbor-joining phylogenetic trees either including or not the reference strains from this web application. If the SIT information was not available and neither the octal nor the binary spoligotyping pattern was described in the article, the strain was not used in this phylogenetic analysis.

Results

In the survey conducted in Salvador, Bahia, only one case of EAI (0.3%) was identified among the 351 successfully genotyped isolates. This corresponded to a 31-year-old

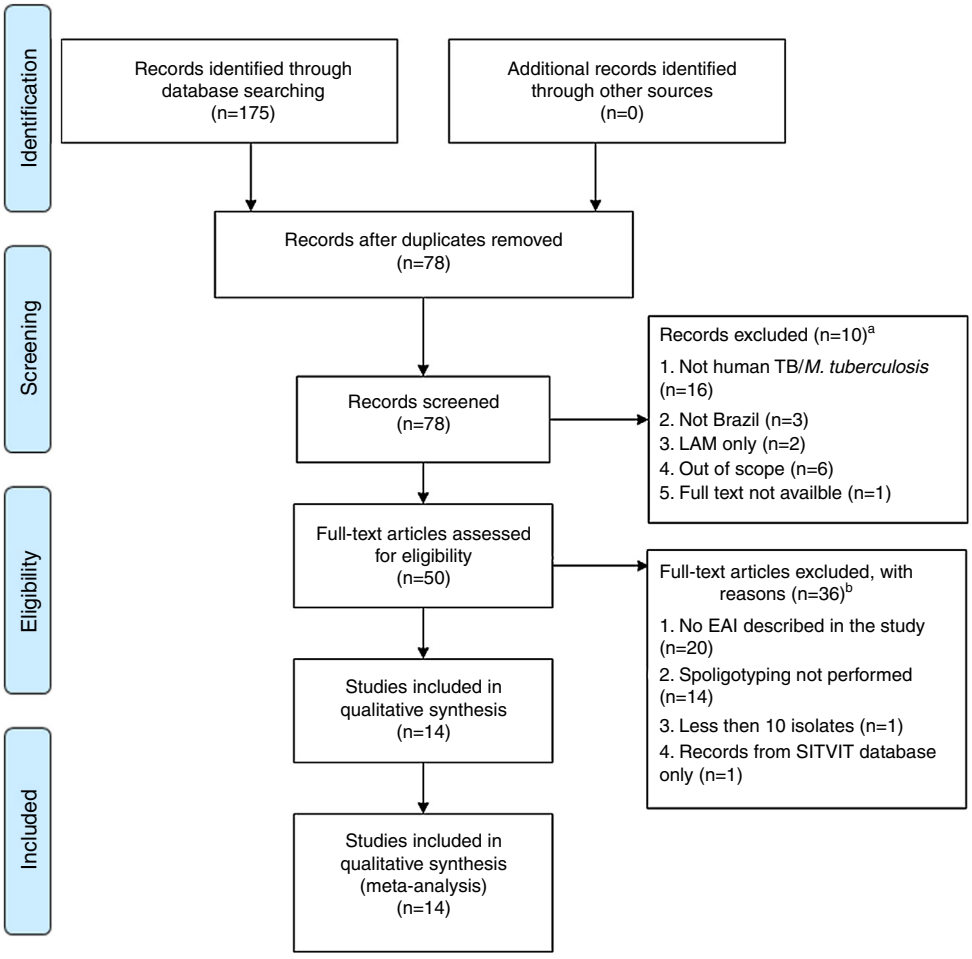

Fig. 2 – PRISMA[31] flow diagram describing the systematic literature review performed. [a]Records screened were excluded after reading the title and abstract if: (i) the study did not focus on *M. tuberculosis*; (ii) the isolates were not identified in Brazil; (iii) the strains analyzed were restricted to a non-East African-Indian (EAI) family of *M. tuberculosis*; (iv) the study did not focus on isolates from humans; (v) the full text was not available via the CAPES Consortium, or access to the article was not provided by Fiocruz. [b]Full-text articles were not included if: (i) they did not report EAI; (ii) spoligotyping was not performed; (iii) less than 10 isolates were described; (iv) the study analyzed records exclusively from the SITVIT database.

diabetic male presenting characteristic symptoms of TB: cough, hemoptysis, night sweats and weight loss. This patient was interviewed and reported that he had never changed residence, nor traveled outside the metropolitan area. He also reported that, in his adolescence, he had contact with a visiting relative from Italy who exhibited typical TB symptoms. The isolate retrieved from this patient was assigned to the EAI6-BGD1 SIT 702 clade in accordance with the spoligotyping profile observed (Fig. 1B). This unique EAI strain was identified by 15 of the 59 SNPs investigated in our series (Table 1, Fig. 1A). Lopes et al.[12] showed that although 10 of these polymorphisms occur in other lineages, the presence of five SNPs: Rv 0629c_0870, Rv 1020_0256, Rv 2362c_0606, Rv 3644c_0726, and Rv 3644c_0735, serves as confirmation of EAI. The RFLP pattern showed 12 bands (Fig. 1C) and was unique in our series (data not published).

Our systematic review of the literature regarding EAI occurrence in studies of Mtb diversity performed in Brazil yielded 175 articles, of which 14 were considered eligible for analysis (Fig. 2 and Table 2).[14–27] Most of these were either based on bacterial collections maintained in reference laboratories that perform culturing for species identification and phenotypic drug-susceptibility testing,[14,16–18,20,21,24,26,27] or on convenience sampling of TB patients at reference health care units responsible for TB diagnosis.[15,22,23,25] One report consisted of a case-control study involving drug-sensitive versus drug-resistant TB patients.[19]

The EAI family is rare in Brazil, occurring typically at frequencies below 2% of the datasets analyzed (Table 2). While EAI family isolates were found in the North,[22,24] Northeast,[19,22] South,[14,16,26] and Southeast of Brazil,[15,17,18,20–23,27] the highest circulation of this family was reported in the North (Pará)[22] (Table 2). The number of EAI isolates varied from only one to as many as 15 in the studies reviewed (Table 2).

The EAI subfamilies most frequently reported in Brazil were EAI6-BGD1 (especially in Pará,[22,24] SIT 129) and EAI5 (SIT 1983) (Fig. 3). Some orphan patterns similar to EAI6-BGD1 were also described in two studies performed in Pará (Fig. 3A). Strains of the EAI5 subfamily SIT 1983 were consistently reported (Table 2 and Fig. 3A).[14,20–23] Furthermore, the spoligotyping patterns EAI3-IND SIT 11 and EAI1-SOM SIT 48 were also present in more than one study (Table 2 and

Table 2 – Literature review summary of East African-Indian (EAI) isolates described in studies of *Mycobacterium tuberculosis* diversity performed in Brazil.

Study period	Study site	Nr. of EAI isolates (%)	SIT	Spoligotype subfamily	Octal pattern	Refs.
1995-2003	Paraná	1 (14.3)	1427	EAI5	457001777413771	16
1997-2005	Rio de Janeiro	1 (0.1)	11	EAI3-IND	477777777413071	22
1997-2005	Pernambuco	1 (1.3)	1983	EAI3-IND	474000377413031	22
1997-2005	Pará	15 (8.1)	48 (N=4)	EAI1-SOM	777777777413731	22
			702 (N=4)	EAI6-BGD1	700775747413771	
			2543 (N=2)	EAI5	773601757013371	
			129	EAI6-BGD1	700777747413771	
			924	EAI5	777600007413371	
			Orphan	EAI5	777600007413771	
			Orphan	EAI6-BGD1	700776747413771	
			Orphan	EAI6-BGD1	700677747413771	
1998-2007	Espírito Santo	1 (0.2)	1983	EAI5	474000377413031	18
2000-2003	Pará	11 (15.3)	Orphan	Orphan	437777777413071	24
			Orphan	Orphan	500777747413771	
			129 (N=5)	EAI6-BGD1	700777747413771	
			Orphan	Orphan	700677747413771	
			Orphan	EAI6-BGD1 /EAI1-SOM[a]	777777747413731	
			Orphan (N=2)	EAI5	777600007413771	
2000-2010	Espírito Santo	1 (0.2)	1983	EAI5	474000377413031	20
2001-2002	São Paulo	1 (2.5)	48	EAI1-SOM	777777777413731	17
2002-2003	Rio de Janeiro	1 (0.3)	11	EAI3-IND	477777777413071	27
2004	Minas Gerais	1 (0.9)	1983	EAI3-IND	474000377413031	23
2005-2006	Rio Grande do Sul	1 (2.0)	1435	EAI1-SOM	774777777413731	26
2005-2008	Paraná	1 (1.4)	48	EAI1-SOM	777777777413731[b]	25
2006-2008	São Paulo	4 (0.5)	Orphan	Orphan	777617705413371	21
			11	EAI3-IND	477777777413071	
			1983 (N=2)	EAI5	474000377413031	
2007-2008	Fortaleza	2 (1.7)	NA	EAI5[c]	NA	19
2008-2009	Rio de Janeiro	1 (0.5)	Orphan	EAI	777741777413600	15
2010-2011	Santa Catarina	10 (2.6)	1983	EAI5	474000377413031	14
			Orphan (N=9)	EAI5[c,d]	777777607700171	

NA, not available.
[a]Ambiguous.
[b]Pattern retrieved from the SITVIT WEB based on the SIT informed by the authors.
[c]As described by the authors.
[d]No match was found in the SITVIT WEB database; the closest matches found in the MIRU-VNTR*plus* database pertain to the LAM family.

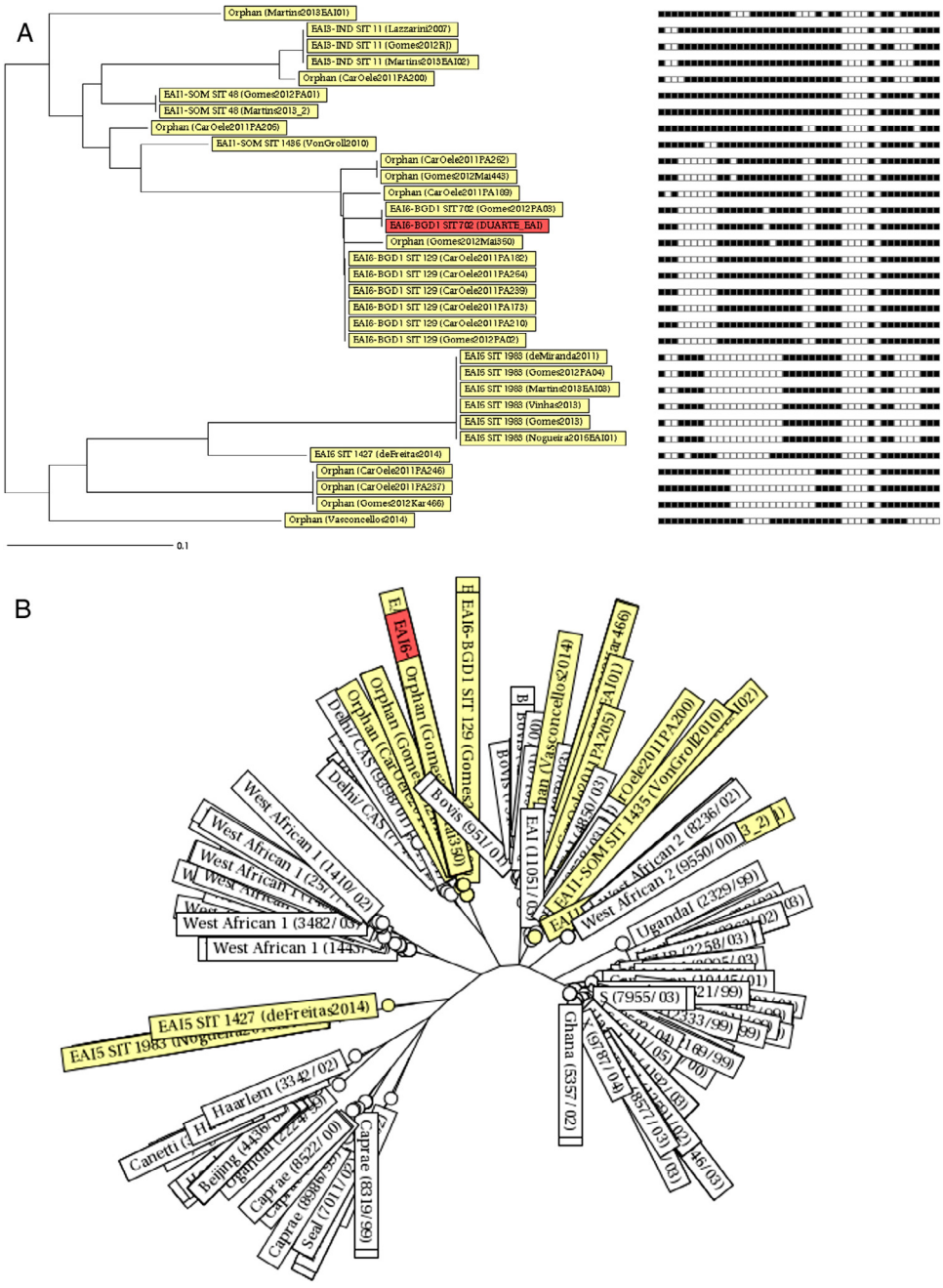

Fig. 3 – Neighbor-joining trees depicting similarities between the East African-Indian (EAI) spoligotyping patterns in Brazil retrieved from the systematic review of the literature and the spoligotyping pattern described in Salvador, Bahia. (A) Dendrogram with corresponding binary spoligotyping patterns. (B) Radiation tree including the reference strains from the MIRU-VNTR*plus* database.

Fig. 3A). These spoligotyping patterns are more similar to other EAI reported in the MIRU-VNTR*plus* database,[28,29] while the EAI6-BGD1 isolates described in Pará and Bahia cluster with the Delhi/Central Asian (CAS) spoligotype (Fig. 3B).

Discussion

The low frequency of EAI in Brazil suggests lower transmissibility of this phylogeographic group than what is observed in other Mtb families. Moreover, other authors have argued that immigration has resulted in the steady influx of particular EAI strains, which has been identified throughout Brazil. On the other hand, this broad distribution, taken together with the restricted genetic variability of the EAI isolates identified in the country, may indicate the endemic nature of this family, albeit at a low prevalence.

The most prevalent subfamilies found in Brazil were EAI6-BGD1 and EAI5. Despite the fact that these subfamilies include spoligotype patterns that are common worldwide, the

EAI6-BGD1 SIT 702 and the EAI5 SIT 1983 clades described herein have restricted circulation outside Brazil. The SITVIT WEB database contains 21 isolates with the EAI6-BGD1 SIT 702 pattern, three of which are Brazilian samples from an outbreak in Pará.[7,22] The remaining specimens were isolated in Cuba, French Guiana (from a patient of Brazilian origin), the United Kingdom, Malawi, Tunisia, and Zambia.[7] For SIT 1983, the clade with the widest distribution in Brazil, the SITVIT WEB database contains data only from Brazil and India.[7] Moreover, strains of the EAI6-BGD1 family (as well as orphan spoligotyping patterns similar to this subfamily, so far not described in the SITVIT WEB database) were previously reported in an outbreak in Pará,[22,24] as well as in our series in Salvador, Bahia, which is indicative of ongoing transmission not restricted to a particular setting. While the EAI6-BGD1 SIT 129 pattern described in Pará has broader worldwide distribution, as isolates from this clade have been previously reported outside Brazil in Germany, the Republic of Congo, Malawi, Zimbabwe, South Africa, Zambia, French Guiana, and the United States,[7] it was also identified in the context of the Pará outbreak. Finally, other widely distributed EAI clades, such as EAI3-IND SIT 11 and EAI1-SOM SIT 48[7] (as well as highly similar orphan patterns) were exclusively found in studies performed in southeastern Brazil. Taken together, these findings suggest that some specific clades of EAI may be better adapted to particular Brazilian populations.

Interestingly, EAI6-BGD1 and similar orphan spoligotyping patterns described in Salvador-Bahia and in the state of Pará, as well as the isolates obtained from the outbreak that occurred in this state, cluster with some strains of lineage 3 (defined by the combined deletion of the genomic regions TbD1 and RD750, including the Delhi/Central Asian (CAS) spoligotyping family[2,5–7]). Lineage 3 belongs to a group of modern Mtb lineages considered to be more virulent than EAI.[2,5–7] Nonetheless, this finding should be interpreted with caution, due to the limited capacity of spoligotyping to accurately distinguish among monophyletic groupings of Mtb.[30]

Conclusions

In spite of the low detected prevalence, EAI may in fact be endemic in Brazil. The restricted worldwide distribution of some spoligotyping patterns described in multiple studies conducted in Brazil, together with the genetic relatedness found among isolates from different parts of the country and the occurrence of an outbreak in Pará, seem to suggest the increased fitness exhibited by some clades with respect to our population.

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Conflicts of interest

The authors declare no conflicts of interest.

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